

REMARKS

Claims 1-7, and 9-19 are pending in the instant application. Claims 8 and 20 have been cancelled, and Applicants reserve the right to prosecute that subject matter, as well as the originally presented claims, in later applications. Claims 1, 2, 15 and 17 have been amended to correct a typographical error. Applicants have also amended claims 1, 15 and 17 to recite “a randomized bridging domain comprising a random nucleotide sequence.” Support for this amendment can be found throughout the specification, and specifically, at least at page 6, lines 4-6, and at page 25, lines 5-13. Applicants have also amended claims 1, 15 and 17 to recite a “pre-existing actuator nucleotide sequence” and/or “a pre-existing receptor nucleotide sequence.” Support for this amendment can be found throughout the specification, and at least at page 23, lines 6-8. Thus, the amendments made herein are fully supported by the specification, and no new matter has been added.

I. Specification

The Examiner has objected to the abstract of the disclosure, because the originally filed abstract was longer than 150 words. Applicants have amended the abstract herein, and as amended, the abstract is not longer than 150 words. Accordingly, this objection has been rendered moot, and Applicants respectfully request that the Examiner withdraw this objection.

II. Drawings

The Official draftsman has objected to the drawings in the present application. In particular, the draftsman has objected to the numbers and reference characters of Figures 5A-5D and 9B, as not plain and legible. Applicants have amended these figures to ensure that the numbers and reference characters are plain and legible. Accordingly, Applicants submit herewith substitute sheets for Figures 5A-5D (p. 7/19 of the drawings) and Figures 9B and 9C (p. 11/19 of the drawings).

The Official draftsman has also objected to Figures 2A-2C as containing the “print-off of another sheet onto FIG. 2A-2C.” Applicants submit herewith a substitute sheet for Figures 2A-2C (p. 2/19 of the drawings), in which the “print-off from another sheet” has been removed

from the left margin.

Applicants respectfully request that pages 2/19, 7/19 and 11/19 of the originally filed drawings be replaced with the substitute drawing sheets (p. 2/19, 7/19 and 11/19) submitted herewith.

III. Information Disclosure

The Examiner has indicated that the Information Disclosure Statement filed on November 27, 2001 has not been considered, because the references have become separated from the application file. The Examiner is encouraged to contact the undersigned if she would like courtesy copies of the references previously submitted in the November 27, 2001 Information Disclosure Statement.

IV. Claim Rejections – 35 U.S.C. § 102

The Examiner has rejected claims 1-7, 9, 10, 12-16 and 19 under 35 U.S.C. §102(a) as being anticipated by Araki *et al.*, *Nucleic Acids Research*, vol. 26(14): pp. 3379-3384 (1998) ("Araki"). In particular, the Examiner has asserted that "Araki et al. teach an allosteric ribozyme using a hammerhead ribozyme as the active site and a flavin-specific RNA aptamer as a regulatory site," and "six variants with a series of base pairs in the linker region (stem II)." (Office Action, p. 6).

Applicants traverse. As amended herein, the claims of the present invention are directed to methods and compositions relating to functional polynucleotides having at least three domains: an actuator domain, a receptor domain and a *randomized* bridging domain, wherein the bridging domain includes *a random nucleotide sequence*. In particular, claim 1 of the present invention is directed to a functional polynucleotide having an actuator domain that contains at least a portion of a pre-existing (*i.e.*, known) actuator, a receptor domain that contains at least a portion of a pre-existing (*i.e.*, known) receptor, and a randomized bridging domain, such that an interaction between the receptor domain and a signaling agent triggers a conformational change in the randomized bridging domain. This conformational change allows the randomized bridging domain to modulate the activity of the actuator domain.

Claim 15 recites a process for preparing the polynucleotides of the present invention, *i.e.*,

polynucleotides that are responsive to a signaling agent. This process includes the linking of an actuator domain having at least a fragment of a known actuator, a receptor domain and a randomized bridging domain, such that interaction between the receptor domain and a signaling agent triggers a conformational change in the randomized bridging domain. Again, this conformational change permits the randomized bridging domain to modulate the activity of the actuator domain.

Claim 17 is directed to a process for screening polynucleotides that are responsive to a signaling agent, wherein the polynucleotides contain an actuator domain, a receptor domain, and a randomized bridging domain. This process comprises the steps of linking (i) an actuator domain having defined properties and containing at least a portion of a known actuator, (ii) a randomized bridging domain that has defined properties and is able to modulate the activity of the corresponding actuator domain and (iii) a receptor domain having a random sequence, and subsequently identifying polynucleotides that are responsive to the signaling agent.

Araki, in contrast, fails to disclose or suggest polynucleotides having a randomized bridging domain that includes a *random nucleotide sequence*. The allosteric ribozymes disclosed by Araki contain a known aptamer (*i.e.*, the FMN binding loop), a known ribozyme (*i.e.*, the hammerhead ribozyme), and a rationally designed linking region (*i.e.*, one of the six constructs disclosed in Figure 1b). Araki explicitly discloses that these six linking region constructs are not random nucleotide sequences. Rather, these constructs were specifically designed to contain the U-A base pair of the FMN-binding loop and a series of G-C base pairs, as “the FMN aptamer was previously found to have conformational characteristics involving formation of a short helix of A-U/G-C pairs on ligand binding.” (See p. 3380, col. 2, “Results and Discussion,” first paragraph). Thus, the linking regions of Araki allosteric ribozymes were designed to contain an A-U base pair and one or more G-C base pairs to mimic the short helix of A-U/G-C pairs that occurs when a ligand binds to the FMN aptamer.

Thus, the linking regions of the Araki allosteric ribozymes were rationally designed based on the known structure and function of the FMN aptamer. Accordingly, this reference fails to teach or suggest a functional polynucleotide having an actuator domain, a receptor domain and a randomized bridging domain, wherein the bridging domain is a *random nucleotide sequence*. Claims 1-7, 9, 10, 12-16 and 19 are not anticipated by Araki, and Applicants, therefore, request

that the Examiner withdraw this rejection.

Claims 1-7, 9, 10, 12-16 and 19 also stand rejected under 35 U.S.C. §102(b) as being anticipated by Tang *et al.*, *Chemistry and Biology*, vol. 4(6): pp. 453-59 (1997) (“Tang”). According to the Examiner, “Tang et al. taught generation of allosteric ribozymes from a hammerhead ribozyme such that the stem II region was modified to contain a region containing an ATP aptamer.” (Office Action, p. 7).

Applicants traverse this rejection. As discussed above, the claims of the present invention are directed to methods and compositions involving functional polynucleotides having at least three domains: an actuator domain, a receptor domain and a *randomized* bridging domain, wherein the bridging domain includes *a random nucleotide sequence*.

The allosteric ribozymes disclosed by Tang, in contrast, are created using “rational design strategies.” The Tang ribozyme constructs contain a known aptamer (*i.e.*, the ATP-binding aptamer), a known ribozyme (*i.e.*, the hammerhead ribozyme), and a rationally designed linking region (*i.e.*, the seven linking region constructs (H1-H7) disclosed in Table 1).

Constructs H1-H7 do not include a random nucleotide sequence. The constructs labeled H1 and H2 contain the known stem II region of the hammerhead ribozyme, while constructs H3 and H4 include the known stem II region of the ATP-aptamer. Construct H5 was specifically designed to include a 3 base-pair extension within the stem II region of the ATP-aptamer. Thus, construct H5 is *not* a randomized linking region. Moreover, Tang explicitly teaches that constructs H6 and H7 were *specifically designed* to replace four base pairs in the stem II region of the polynucleotide with “less stable G-U mismatches.” (See p. 456, col. 2, second paragraph). In fact, Tang acknowledges “we intended to exploit the fact that the G-C pair that begins stem II within the aptamer domain is not paired in the absence of ATP, but will form a stable pair when ATP is complexed.” (*Id.*) The linking regions taught by Tang include either previously known sequences, or the linking regions include sequences that have been rationally designed using previous knowledge of the structure and function of the ATP aptamer.

Thus, Tang fails to teach or suggest a functional polynucleotide having an actuator domain, a receptor domain and a *randomized* bridging domain, wherein the bridging domain is *a random nucleotide sequence*. Accordingly claims 1-7, 9, 10, 12-16 and 19 are not anticipated by Tang, and Applicants request that the Examiner withdraw this rejection as well.

V. Claim Rejections – 35 U.S.C. § 103(a)

The Examiner has rejected claims 11, 17 and 18 under 35 U.S.C. §103(a) as being unpatentable over Araki, Tang, and Breaker *et al.*, *Chem. Rev.*, vol. 97: pp. 371-390 (1997) ("Breaker"). According to the Examiner, Araki and Tang do not teach that the polynucleotides disclosed therein can be attached to a solid support, and furthermore, these references fail to disclose a polynucleotide "wherein the receptor domain has a random sequence." (Office Action, p. 9). In particular, the Examiner asserts:

It would have been *prima facie* obvious at the time the invention was made to one of ordinary skill in the art to attach the polynucleotides taught by Araki *et al.* or Tang *et al.* to a solid support as taught by Breaker *et al.* for the purpose of catalytic elution of the reacted ribozyme since Breaker *et al.* taught that such methods of using a solid support for the isolation of the catalytic molecule was another type of selection to the gel isolation type of selection used by Araki *et al.* for example. It would have been *prima facie* obvious to one of ordinary skill in the art to practice a method of optimizing the allosteric ribozymes taught by Tang *et al.* or Araki *et al.* with a method of screening comprising use of randomized ribozyme sequence since Tang *et al.* taught generally the use of *in vitro* screening techniques for optimization of the allosteric ribozymes (page 457 and page 458) in addition to the examples of rational screening, and Breaker taught that *in vitro* evolution experiments often use randomized sequences as an 'alternative (or complement) to rational design...' (Office Action, pp. 9-10).

Applicants traverse. Claim 11 indirectly depends from independent claim 1, and therefore, necessarily contains all of the limitations recited by claim 1. As described above, claim 1 is directed to a functional polynucleotide having an actuator domain that contains at least a portion of a pre-existing (*i.e.*, known) actuator, a receptor domain that contains at least a portion of a pre-existing (*i.e.*, known) receptor and a randomized bridging domain, such that an interaction between the receptor domain and a signaling agent triggers a conformational change in the randomized bridging domain. This conformational change allows the randomized bridging domain to modulate the activity of the actuator domain.

Claim 17 is directed to a process for screening polynucleotides that are responsive to a signaling agent, wherein the polynucleotides contain an actuator domain, a receptor domain, and a randomized bridging domain. This process comprises the steps of linking (i) an actuator domain having defined properties and containing at least a portion of a known actuator, (ii) a

randomized bridging domain that has defined properties and is able to modulate the activity of the corresponding actuator domain and (iii) a receptor domain having a random sequence, and subsequently identifying polynucleotides that are responsive to the signaling agent. As recited by dependent claim 18, the receptor domain can further include a ligand-binding site, such that ligand binding triggers a conformational change in the randomized bridging domain. This conformation change allows the randomized bridging domain to stimulate the catalytic activity of the actuator domain.

As discussed above, neither Araki nor Tang discloses a functional polynucleotide having an actuator domain, a receptor domain and a *randomized* bridging domain that includes a random nucleotide sequence. In these references, the linking regions were rationally designed using the known structure and function of the ATP aptamer and the FMN aptamer. There is no teaching or suggestion in these references, either alone or in combination, that would motivate one of ordinary skill in the art to modify the polynucleotides disclosed by Araki and Tang to produce an actuator domain that includes at least a portion of a known actuator, coupled to a receptor domain via a randomized bridging domain having a random nucleotide sequence.

Araki

There is no teaching or suggestion in Araki that the disclosed polynucleotide constructs can include a randomized linking region and still remain functional. Each of the linking regions disclosed by Araki was rationally designed based on the known structure and function of the FMN aptamer. The Examiner has acknowledged that the teachings and suggestions in Araki are limited to rationally designed ribozyme constructs. According to the Examiner, “Araki et al. taught motivation to *design* of [sic] different ribozymes for isolation of the best catalytic sequence.” (Office Action, p. 10, emphasis added). As one of ordinary skill in the art would not be motivated by Araki to produce the disclosed allosteric ribozyme constructs using *any* method other than rational design, claims 11, 17, and 18 are not obvious in view of Araki.

Tang and Breaker

Additionally, one of ordinary skill in the art would not be motivated by the teachings of Tang and Breaker, either alone or in combination, to produce a polynucleotide having an actuator domain that includes at least a fragment of a pre-existing (*i.e.*, known) actuator nucleotide

sequence linked to a receptor domain via a randomized linking region. At p. 458, col. 1, Tang discusses refining the interplay between the aptamer and ribozyme motifs to improve ribozyme catalytic rates. According to Tang, these improvements can be made using “a combinatorial library of RNAs followed by screening via *in vitro* selection.” To support this disclosure, Tang cites Breaker. However, Tang fails to disclose or suggest any other methods of refining the interplay between the aptamer and ribozyme motifs of the disclosed allosteric ribozyme constructs.

The *in vitro* screening methods taught by Breaker are used to isolate novel catalytic nucleic acids from pools of completely random RNA sequences. According to Breaker, this approach “relies on the probability that a given pool of random sequence molecules will include individuals that can perform the function of interest” (*i.e.*, catalytic activity). (Breaker, p. 372, col. 2). In other words, the *in vitro* selection methods taught by Breaker (and Tang by reference) identify fully randomized ribozyme sequences.

Combining the randomized ribozyme sequences taught by Breaker (and Tang) with the allosteric ribozyme constructs disclosed by Tang does not, however, teach or suggest the claimed polynucleotide constructs, which contain an actuator domain that has at least a portion of a pre-existing (*i.e.*, known) actuator domain coupled to a receptor domain via a randomized bridging domain. Rather, the combination of Breaker and Tang teaches an allosteric ribozyme construct having a randomized actuator domain (*i.e.*, the randomized ribozymes identified by the Breaker *in vitro* screening methods) coupled to the ATP aptamer (*i.e.*, the receptor domain) via a rationally designed linking region.

One of ordinary skill in the art would not be motivated by this combination to arrive at the polynucleotides of claimed invention. Tang discloses using the *in vitro* screening methods of Breaker to refine the interplay between the aptamer and ribozyme motifs of the disclosed constructs, but fails to teach or suggest any other methods of optimizing the catalytic activity of the disclosed allosteric ribozyme constructs. To suggest that it would have been obvious to optimize the interplay between the aptamer and ribozyme motifs by another method, such as, for example, by using a randomized bridging domain, would be an improper application of hindsight.

As one of ordinary skill in the art would not have been motivated by the teachings of Tang

and Breaker, either alone or in combination, to modify the disclosed allosteric ribozyme constructs to arrive at the claimed invention, claims 11, 17 and 18 are not obvious in view of these references.

Accordingly, Araki, Tang and Breaker, either alone or in combination, fail to render the claimed invention obvious, and Applicants request that the Examiner withdraw this rejection of claims 11, 17 and 18.

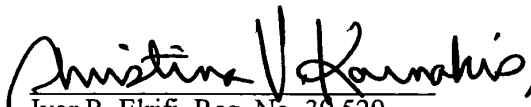
CONCLUSION

On the basis of the foregoing amendments and remarks, Applicant respectfully submits that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 23239-201 NATL.

Respectfully submitted,

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Version with Markings to Show Changes Made

In the specification:

The abstract has been replaced with the following rewritten paragraph:

Multidomain polynucleotides, which are responsive to [signalling] signaling agents [are designed and constructed to] and have at least three domains which can be partially or completely overlapping or nonoverlapping: an actuator (catalytic or reporter) domain, a bridging domain, and a receptor domain, are provided. In a typical embodiment, a [signalling] signaling agent [such as a chemical ligand] interacts with the receptor domain, which changes conformation or otherwise influences the bridging domain so that the [activity,] catalytic[,] or reporter function of the actuator domain is stimulated or inhibited. In some ribozyme embodiments, [for example,] ligand-specific molecular sensors composed of RNA are created by coupling [pre-existing] preexisting catalytic and receptor domains via novel structural bridges, wherein [which function such that] binding of a ligand to the receptor domain triggers a conformational change within the bridge, and this structural reorganization dictates the activity of the adjoining ribozyme. Processes for allosterically selecting other multidomain polynucleotides typically involve mixing and matching domains to optimize binding or other signal response and/or reporter activity.

In the claims:

Claims 8 and 20 have been cancelled.

Claims 1, 2, 15 and 17 have been amended as follows:

1. (Amended) A purified functional polynucleotide comprising
 - (a) an actuator domain comprising at least a fragment of a pre-existing actuator nucleotide sequence,
 - (b) a receptor domain comprising at least a fragment of a pre-existing receptor nucleotide sequence, and
 - (c) a randomized bridging domain comprising a random nucleotide sequence,
wherein interaction of the receptor domain with a [signalling] signaling agent triggers a conformational change in the randomized bridging domain which modulates the activity of the

actuator domain.

2. (Amended) A polynucleotide according to claim 1 wherein the ~~[signalling]~~ signaling agent is a ligand that binds to the receptor domain.

8. (Cancelled)

15. (Amended) A process for preparing polynucleotides that are responsive to the presence or absence of a ~~[signalling]~~ signaling agent, comprising linking together a polynucleotide actuator domain comprising at least a fragment of a pre-existing actuator nucleotide sequence, a receptor domain comprising at least a fragment of a pre-existing receptor nucleotide sequence, and a randomized bridging domain [together] comprising a random nucleotide sequence, such that interaction of the ~~[signalling]~~ signaling agent with the receptor domain triggers a conformational change in the randomized bridging domain which modulates the activity of the actuator domain.

17. (Amended) A process for screening polynucleotides which have an actuator domain, a receptor domain, and a randomized bridging domain and which are responsive to a ~~[signalling]~~ signaling agent in a sample, comprising linking a randomized bridging domain comprising a random nucleotide sequence and having defined properties that modulate the activity of a corresponding actuator domain having defined properties and comprising at least a fragment of a pre-existing actuator nucleotide sequence, to a receptor domain having a random sequence, and identifying polynucleotides responsive to the ~~[signalling]~~ signaling agent by ~~[incubation of]~~ incubating the sample with the polynucleotide so constructed and by ~~[observation of]~~ observing modulation of the activity of the actuator domain.

18. (Amended) A process according to claim 17 wherein the receptor domain has a ligand binding site and wherein ligand binding triggers a conformational change in the randomized bridging domain that stimulates catalytic activity of the actuator domain.

20. (Cancelled)